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[COMMUNICATION]

Hatching Patterns of the Monogenean Parasites *Benedenia seriolae* and *Heteraxine heterocerca* from the Skin and Gills, Respectively, of the Same Host Fish, *Seriola quinqueradiata*

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ABSTRACT—Eggs of the capsalid monogenean skin parasite *Benedenia seriolae* and the polyopisthocotylean gill parasite *Heteraxine heterocerca,* from the same host, the yellowtail *Seriola quinqueradiata,* have strikingly different hatching patterns when incubated under identical environmental conditions with natural illumination and constant temperature. *B. seriolae* eggs hatch throughout the hours of daylight, with an indication of an early and a late hatching peak during the day, while *H. heterocerca* has a strong hatching peak at dusk and emergence declines in frequency during the night. The significance of this difference in hatching pattern is discussed.

INTRODUCTION

It has been found by one of us (YM) that the eggs of two unrelated monogenean parasites, namely *Benedenia seriolae* (Yamaguti, 1934) Price, 1939 and *Heteraxine heterocerca* (Goto, 1894) Yamaguti, 1938, from the skin and gills respectively of the yellowtail, *Seriola quinqueradiata,* readily become entangled in nylon netting suspended in the tank with the fishes. This provides an excellent opportunity to incubate and hatch the eggs of the two parasites under identical environmental conditions and, since the larvae of the two parasites can readily be distinguished with a dissecting microscope, to compare their daily hatching patterns. This is important because there has been only one comparative study of hatching in monogeneans sharing the same host fish, namely that of Whittington [1] and although *S. quinqueradiata* is an important food fish in Japan, cultured extensively in marine fish farms, the hatching patterns of *B. seriolae* and *H. heterocerca* are unknown.

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MATERIALS AND METHODS

Eggs of *Benedenia seriolae* and *Heteraxine heterocerca* became entangled by their appendages in nylon netting with a mesh size of about 1.5 mm, suspended in tanks containing infested fishes at the National Research Institute of Aquaculture at Nansei. The netting was left *in situ* for about 24 hr and the following day transported to the Seto Marine Biological Laboratory at Shirahama. Two pieces of the netting about 40 cm^2 were cut out and each piece was placed in a crystallizing vessel with sloping sides and a bottom diameter of about 6 cm containing about 2 cm of filtered sea water. The vessels were incubated at 23°C in a glass-fronted constant temperature cabinet facing a north window. The approximate official times of dawn and dusk in the Shirahama area at the time of year (October) when the experiments were conducted were 06.00 hr and 17.30 hr respectively. During

the period of incubation the sea water was changed twice a day by gently transferring each piece of netting with forceps to a new vessel. When free-swimming larvae were detected, the netting was transferred in the same way to fresh sea water at 2-hourly intervals throughout the day and the night. During the night a dim background light permitted detection and transfer of the white netting. After each transfer, a few drops of formaldehyde were added to the vessel from which the netting had been removed. This treatment killed rapidly any free-swimming larvae and since dead oncomiracidia of *B. seriolae* and *H. heterocerca* are easy to distinguish from each other with a stereomicroscope by their size and shape (Fig. 1), it was possible to determine the number of oncomiracidia of each species which had hatched during each 2 hr period. Counting was made easier by inscribing a grid on the bottom of each glass vessel and, since larvae occasionally become trapped at the water/air interface, it was also necessary to scan the surface of the sea water in each vessel.

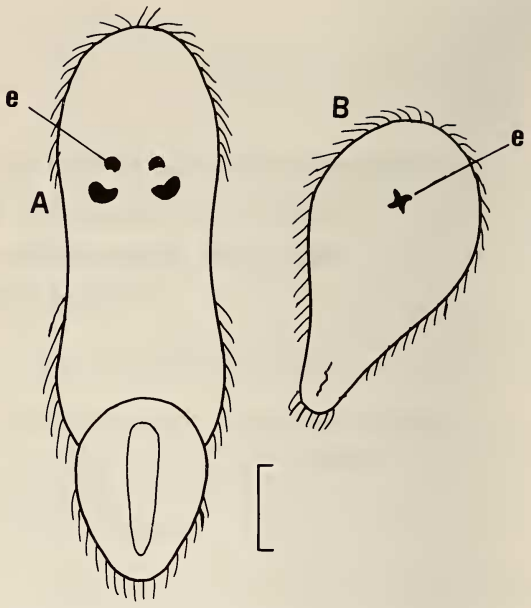

Fig. 1. The shapes and main features of preserved oncomiracidia of A, *Benedenia seriolae* and B, *Heteraxine heterocerca*. e, Eye. Scale bar=50 μm.

RESULTS

Oncomiracidia were first discovered in the vessels on the fifth day after the beginning of the egg collection period. Regular sampling at intervals of 2 hr was begun immediately and continued without interruption until no more larvae were available for hatching (on the fourth day of recording in *B. seriolae* and on the second day in *H. heterocerca*). The hatching patterns for the two parasites from

Table 1. Numbers of larvae of *Benedenia seriolae* and *Heteraxine heterocerca* collected at 2 hr intervals beginning on Day 1.

Time	*B. seriolae*				*H. heterocerca*		
	Day 1	Day 2	Day 3	Day 4	Day 1	Day 2	Day 3
04.00–06.00 (dawn 06.00)	—	81	94	12	—	2	0
06.00–08.00	—	467	214	16	—	0	0
08.00–10.00	—	214	173	5	—	0	0
10.00–12.00	—	209	22	3	—	1	0
12.00–14.00	2	282	79	1	9	0	0
14.00–16.00	14	943	48	2	2	0	0
16.00–18.00 (dusk 17.30)	38	408	24	0	187	107	0
18.00–20.00	11	58	1	0	109	13	0
20.00–22.00	1	12	0	—	18	0	0
22.00–24.00	0	3	0	—	14	0	—
24.00–02.00	0	0	0	—	8	0	—
02.00–04.00	0	0	0	—	5	0	—

The figures are the sums of larvae from two separate experiments. —, no observation made.

the two separate experiments were similar and the results from the two experiments have been added together (see Table 1). The total numbers of larvae of *B. seriolae* and *H. heterocerca* collected were 3437 and 475, respectively. It can be seen from Table 1 that *B. seriolae* larvae hatched throughout the hours of daylight but after dusk hatching was curtailed and few larvae emerged

FIG. 2. The daily hatching pattern of A, *Benedenia seriolae* and B, *Heteraxine heterocerca*. The histogram value for each 2 hr period is the sum of all larvae hatching during the same period on all days when larvae were collected, expressed as a percentage of the total number of larvae collected. Night-time is indicated by the black bar beneath the histogram and the official times of dawn and dusk are shown by the vertical arrows.

during the night. On the second and third days of recording there was an initial period of extensive hatching during the period 04.00 to 08.00 hr, around the time of dawn. Hatching then continued but declined in frequency until about 12.00 hr when it again showed a significant increase until dusk. In *H. heterocerca,* few oncomiracidia were observed during daylight but there was a pronounced hatching peak on the first and second days of recording at dusk; hatching continued with decreasing frequency during the night.

These contrasting patterns can be seen more clearly in Fig. 2, in which the daily hatching patterns for each species are compared by summing all the larvae of each species hatching during the same 2 hr period on each day of observation, and expressing this as a percentage of the total number of larvae collected. This also emphasizes the apparently bimodal nature of the diurnal hatching of *B. seriolae* with peaks at 06.00–08.00 hr and 14.00–16.00 hr.

DISCUSSION

The hatching patterns of the monogeneans *Benedenia seriolae* and *Heteraxine heterocerca,* from the same host, *Seriola quinqueradiata,* have been determined under identical environmental conditions. Nylon netting with the entangled eggs of both parasites was incubated at 23°C with natural illumination, conditions which are not too different from those in the natural environment. If it is assumed that monogeneans hatch at a time of day or night when the host is most vulnerable to infection, then it would be expected that monogeneans such as *B. seriolae* and *H. heterocerca,* which share the same host, would exhibit identical daily hatching patterns. Although there is some degree of convergence between the two parasites in the nature of their egg appendages and the fate of the eggs, their hatching patterns are surprisingly different (Fig. 2); in the skin parasite *B. seriolae* hatching continues throughout the hours of daylight, while the gill parasite *H. heterocerca* has a strong hatching peak at dusk and emerges with declining frequency during the night. A possible explanation for this paradox is that the two parasites have different invasion sites and that access to these different sites may be optimal at different times of day. There is evidence indicating that their invasion sites are different because post-oncomiracidia of *B. seriolae* were collected from the skin of experimentally infected *Seriola aureovittata* 24 hr after exposure to oncomiracidia (Kearn, Ogawa and Maeno, in preparation), while Ogawa and Egusa [2] found very young specimens of *H. heterocerca* without clamps on the gills, indicating that the oncomiracidia of the latter parasite are drawn in with the gill-ventilating current.

There is evidence that other polyopisthocotylean gill parasites invade the gills directly, for example *Rajonchocotyle emarginata* and *Plectanocotyle gurnardi* (see [3] and [4] respectively). Whittington [1] has made a comparison of hatching in two monogeneans, which, like *B. seriolae* and *H. heterocerca,* inhabit the skin and gills respectively of the same host. These are parasites of the dogfish, *Scyliorhinus canicula,* with the skin infected by the microbothriid monogenean *Leptocotyle minor* and the gills by the polyopisthocotylean *Hexabothrium appendiculatum.* He found a striking convergence between the two parasites, with similarities between them in egg shapes and in their requirement for a chemical hatching stimulus from the host. Whittington [5] pointed out that similarities between the two parasites in their oncomiracidial behaviour indicate that the larvae of both parasites establish themselves on the skin, those of *H. appendiculatum* migrating later to the gills. Therefore, although these two parasites occupy different microhabitats when adult, their larvae may share the same invasion site, namely the skin.

The best time for invading the skin is not necessarily the best time for invading the gills and some behavioural features of yellowtail, as yet unknown, may create a situation where establishment of oncomiracidia on the skin is more likely to be successful during the day while access of oncomiracidia to the gills *via* the gill-ventilating current may be more favoured at dusk or early in the night. There may be a difference between the oncomiracidia of *B. seriolae* and *H. heterocerca* in their responses to light, gravity and water currents related to their essentially diurnal and nocturnal

hatching patterns and different invasion sites. Apart from a comment by Hoshina [6] on the positive phototaxis of the larva of *B. seriolae,* the behaviour of these oncomiracidia is unknown and deserves further study.

The arguments above assume that *S. quinqueradiata* is the natural host for *B. seriolae* and *H. heterocerca.* There is little doubt that *S. quinqueradiata* is the natural host of *H. heterocerca* since the parasite was reported from this host by Goto [7], by Yamaguti [8] and, as *Axine seriola,* by Ishii [9]. However, there is no record of *B. seriolae* from *S. quinqueradiata* in the wild. Yamaguti [10] collected his original specimens of *B. seriolae* from the skin of wild *S. aureovittata* and no further host records were reported by Kamegai and Ichihara [11]. Thus, although the parasites share the same host, *S. quinqueradiata,* in fish farms, it is not certain that they do so in the wild and since the behaviour of even closely-related hosts may differ, the hatching patterns of *B. seriolae* may be attuned to a host other than *S. quinqueradiata.*

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REFERENCES

1 Whittington, I. D. (1987) J. mar. biol. Ass. UK., **67**: 729–756.
2 Ogawa, K. and Egusa, S. (1981) Bull. Jpn. Soc. scient. Fish., **47**: 1–7.
3 Whittington, I. D. and Kearn, G. C. (1986) J. mar. biol. Ass. UK., **66**: 91–111.
4 Whittington, I. D. and Kearn, G. C. (1989) J. mar. biol. Ass. UK., **69**: 609–624.
5 Whittington, I. D. (1987) J. mar. biol. Ass. UK., **67**: 773–784.
6 Hoshina, T. (1968) Bull. Off. int. Épizoot., **69**: 1179–1191.
7 Goto, S. (1894) J. Coll. Sci. imp. Unv. Tokyo, **8**: 1–273.
8 Yamaguti, S. (1938) Jpn. J. Zool., **8**: 15–74.
9 Ishii, N. (1936) Zool. Mag., Tokyo, **48**: 781–790.
10 Yamaguti, S. (1934) Jpn. J. Zool., **5**: 241–541.
11 Kamegai, S. and Ichihara, A. (1972) Res. Bull. Meguro Parasit. Mus., **6**: 1–43.